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Uromyces scutellatus as a keystone species affecting *Euphorbia* spp. in Europe as shown by effects on density in the field

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Abstract
Nearest neighbor spatial analysis was used to assess the effect of systemic rust caused by *Uromyces scutellatus* on stand density of *Euphorbia esula/virgata*, a highly invasive deep-rooted perennial weed of rangelands and natural areas in North America. ANOVA applied to nearest neighbor measurements within four pairs of stands in close proximity, with and without rust, in Hungary and Austria indicated that the stand densities of plants of *E. esula/virgata* in three of four rusted stands were less than companion stands with little or no rust. Using the nearest neighbor distance data, *E. esula/virgata* densities within stands where rust was prevalent were 48–73% of those with little or no rust. The fourth stand with rust was denser than a symptomless companion stand in 2004, but nearly all plants had symptoms. The same diseased stand was dramatically reduced in density when surveyed the following year and all plants observed in 2005 displayed rust symptoms. These findings indicate the potential impact of the microcyclic autoecious rust *U. scutellatus* should it be introduced as a biocontrol agent for *E. esula/virgata* in North America.

Keywords: *Classical biocontrol, nearest neighbor spatial analysis, rust disease, pre-release studies, invasive species*

Introduction

Biological control of exotic invasive plants is based on the enemy release hypothesis. In practice, this entails searches within the native range of the invasive for insects or plant pathogens that can damage or cause disease on the target plant species. It is increasingly understood in the field of invasive weed biocontrol that relying solely on narrow-host range is unsufficient as a strategy for selecting agents. This prevailing method of selecting new agents (chiefly insects) has come to be known as the 'lottery approach' (Denoth et al. 2002; Balciunas 2004), with the evident implications that such a term would entail. The author has called for new approaches in selecting agents that lead to fewer, more effective agents (Caesar 2001) based on the propensity of an agent to participate in multitrophic interactions. Recently, others have also contributed to an emerging consensus

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about the need for new approaches with a similar emphasis: to select agents with 'stronger' effects to reduce the risk of negative indirect effects of biocontrol (Pearson & Callaway 2003). This should necessarily apply to both insect and microbial classical agents for weed biocontrol. Until now, studies to assess the potential impact of a candidate agent have been rare and concerned solely with the classical plant pathogen modality of invasive weed biocontrol (Baudoin et al. 1993; Shishkoff & Bruckart 1996) and have failed to become a regular part of assessing potential agents.

Following tests to establish a narrow host range of an agent, a second step for candidate agents qualified this way would be to assess the magnitude of potential impacts prior to field release. In addition to such 'prerelease studies', which typically occur in a greenhouse setting, there should be an assessment of impact in the native range. The potential of candidate biological agents to cause reductions in population density of this species could best be previewed by assessment of their effects in the native environment. This would be most valuable even prior to prerelease studies. This approach should apply to candidate species that are plant pathogens as well as insects. The occurrence of *Euphorbia esula* L./*virgata* (L.) Waldst. & Kit. in widely scattered, sparse stands in Eurasia, documented previously (Caesar et al. 1999) and the irregularity of the patches as to size and shape prevents the application of conventional quadrat-based methods to assess the relative effects of rust on stand density of the weed. Based on these factors it was decided to use plotless methods to estimate the effects of systemic rust disease on *E. esula*/*virgata*. Such methods are especially useful where stands are irregular in size and occur in small patches (Elzinga et al. 2001). Therefore, I have assessed the effects of the autoecious, microcyclic rust fungus *Uromyces scutellatus* (Schrank) Lév. 1847 on stand density of *E. esula*/*virgata* by comparing population densities among stands with and without symptoms using nearest neighbor-based assessments of the comparative distances between plants and plant densities. The present work continues a series of studies by the author that have sought to elucidate mechanisms and impact by candidate agents in their native range (Caesar et al. 1996, 1998, 1999, 2002; Caesar 2003), an aim recommended by Wapshere (1985) in the field of weed biological control which, from a different vantage point has begun to receive greater emphasis in the field of invasive species ecology recently (Hierro et al. 2005).

Beyond introducing a potential new means of assessing the impact of a candidate agent in the field, an equivalent aim of this study was to meet the need for a new agent that could effect biocontrol at sites not yet impacted by insect releases. While successful biological control of leafy spurge, chiefly with *Aphthona* spp. flea beetles, has occurred over large areas of North America, establishment has been variable depending on the individual *Aphthona* species released. This variability has been attributed to host genotype and a wide variety of environmental factors (Lym & Carlson 2002; Nelson & Lym 2003). Sites that have been documented to have poor establishment include sandy soils (Mundal & Carlson 1999). Furthermore, slope and aspect are also considered to affect establishment (Kirby et al. 1999). While a recent study showed that high populations of the flea beetles correlate with stand reductions of *E. esula*/*virgata*, ca. 66% of sites had low numbers of *Aphthona* (Kalischuk et al. 2004), indicating that most sites have not experienced biocontrol. Thus, there remain a large number of sites as yet unaffected by biological control measures (Caesar 2003),

where an agent such as *Uromyces scutellatus* could provide an alternative means to reduce stand density of this invasive perennial.

Materials and methods

To determine whether systemic rust disease caused by *U. scutellatus* is associated with lower stand density of *E. esula/virgata* in their common European native range, locations were sought where stands with symptoms of systemic rust occurred in close proximity to stands of *E. esula/virgata* which were free of rust or which contained no more than two to three plants with rust among at least 100 plants. *Euphorbia* species infected with members of the *U. scutellatus* complex of species are distinguished from other *Uromyces* species by the lack of nectar formation on the 'pseudoflowers' caused by rust infection (Pfunder et al. 2001) and this was a third criterion for the selection of study sites. Identification of candidate sites and confirmation that pairs of stands met these criteria resulted from multiple visits to the prospective sites in Austria and Hungary between 1992 and 2005. Data were gathered in 2004 and 2005 at four sites that met these requirements. Because *E. esula/virgata* occurs in small, irregular stands, the use of 'plotless' methods were chosen as appropriate for analyzing the relative effects on stand density of systemic rust disease. Such methods, using distances to the second or third nearest neighbor (Morisita 1957), do not require a random distribution of sampled units and have been shown be the most effective and robust regardless of the plant distribution pattern among a variety of methodologies tested (Engeman et al. 1994). Sites in Hungary and Austria with populations of plants of *E. esula/virgata* infected by the autoecious, microcyclic rust *U. scutellatus* were measured for the distance from 10 to 20 (measuring distances from more than 20 plants has been shown to yield diminishing returns (Nielson et al. 2004) per amount of effort) arbitrarily chosen plants of *E. esula/virgata* with rust disease symptoms to the second and third nearest three plants to each. Nearby sites where populations were free of rust disease symptoms were assessed the same way: the distances were recorded from 10 to 20 symptomless plants of *E. esula/virgata* to the second and third nearest neighbors. Using ANOVA, effects of both rust and site on distance between plants of *E. esula virgata* were found. Therefore, using ANOVA, the effect of rust was tested separately for each site. Following tests to confirm homogeneity of variances between data sets for both years by location, the distances were analyzed for each of the four sites using one-way ANOVA with JMP version 5.1. Following ANOVA, to describe the effects of rust, density estimates as shoots per m^2 were obtained from nearest neighbor results from each pair of plots using an algorithm for estimating density from measurements of second and third nearest neighbor (Morisita 1957) implemented with the computer program Density from Distance v. 1.1 (Pisces Conservation Ltd., Lymington, UK). Because of the dramatic reduction in stand density that was evident at the site with rust disease at Gyongos, Hungary, the results of 2004 and 2005 at this location are presented separately.

Results

At three of four sites assessed in Hungary and Austria in 2004 and 2005, stands of symptomless *E. esula virgata* plants were significantly more closely spaced than

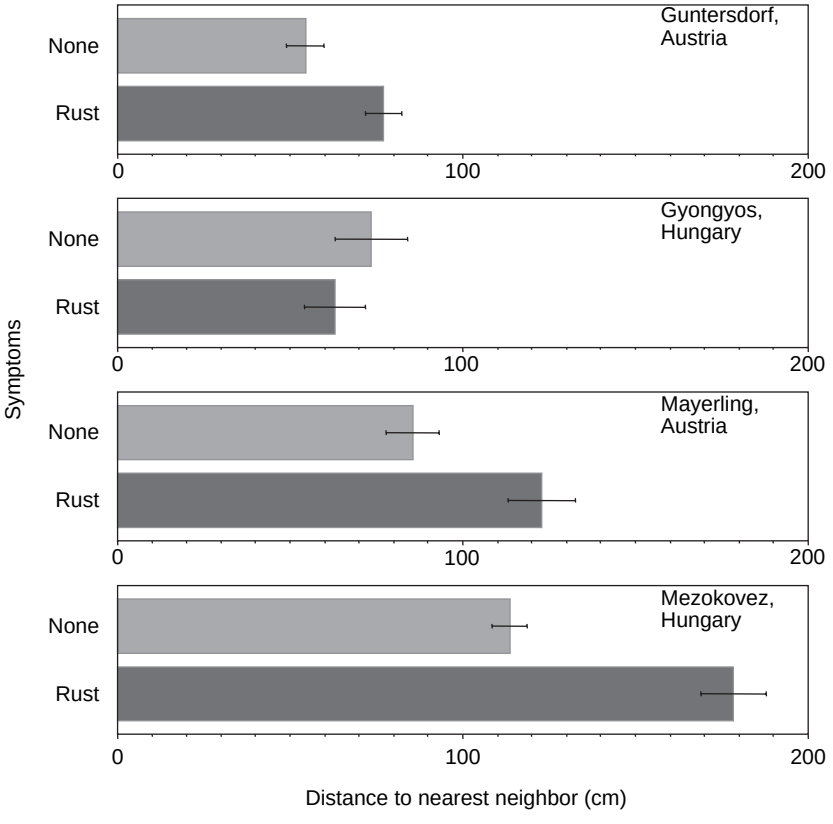

Figure 1. Comparative mean distances between plants of *Euphorbia esula/virgata* within stands with occurrence of rust and nearby stands without rust at four sites in Hungary and Austria in 2004 and 2005.

stands with rust symptoms as determined with ANOVA (Guntersdorf, Austria: rust, $F = 9.35$, df = 1,93, $P = 0.003$; Mayerling, Austria: rust, $F = 7.84$, df = 1,97, $P = 0.006$; Mezokovezd, Hungary: rust, $F = 41.15$, df = 1,166, $P = < 0.0001$; Gyongyos, Hungary: $F = 0.598$, df = 1,41, $P = 0.433$ (Figure 1)). The densities of rusted stands estimated from the distance data were 48–73% of neighboring symptomless stands (Table I). Thus, it appears the rust *U. scutellatus* is associated with lower stand density where it occurs at sites in Europe compared to adjacent or nearby stands with little or no systemic rust disease. At the Gyongyos, Hungary site, while the rusted plants were associated with greater density in 2004, the density was found to be dramatically less in 2005. The author has observed similar situations previously. Disease within stands of *E. esula/virgata* caused by the autoecious, microcyclic rust *U. scutellatus* is associated with at least half the stand densities of adjacent or nearby stands that are symptomless. Heavy levels of infection incidence one year can result in drastic reductions in population density of *E. esula/virgata* in subsequent years. *Uromyces scutellatus* thus exhibits a high level of impact in its native range and if narrow in host range would be an excellent biological control agent for the invasive perennial leafy spurge.

Table I. Comparative stand densities of *E. esula virgata* at four locations in Hungary and Austria where rust disease was present and a nearby stand was disease free. *Euphorbia* density estimated from nearest neighbor analysis.[a]

	Estimated Plant density/m^2	
	Symptoms	
Site	Rust	None
Gyongyos, Hungary[b]		
2004	0.906	0.665
2005	0.220	0.707
Mezokovezd/Rt 3, Hungary[c]	0.113	0.678
Mayerling, Austria[c]	0.238	0.492
Guntersdorf, Austria[c]	0.585	1.214

[a]Plants of *E. esula/virgata* estimated with the algorithm of Morisita (1957). [b]Data from each year are presented separately for this location because of the occurrence of a drastic reduction in density between 2004 and 2005 at this site where severe rust was observed on a dense stand of *E. esula/virgata* in 2004. [c]Mean densities presented for this site are based on data combined from both 2004 and 2005.

Discussion

Analysis of the effects of candidate biological control agents can and should be conducted in the native range of the targeted invasive species. Such 'prerelease' or pre-exploration studies can serve to reduce the hazards of non-target effects due to the 'lottery approach' to biological control by providing a preview of what effects the candidate agent can potentially cause upon release for biocontrol or can be correlated with when assessed against a more complex ecological background than is practical in greenhouse studies. This approach is similar to that outlined by Wapshere (1985), recommending that impacts on the target weed species be assessed in the native range of candidate agents. Such studies would allow identification of keystone species in a less haphazard way than current practices have been conceded to be (Balciunas 2004). As stated recently in a popular work (Mann 2005), 'Keystone species have a disproportionate impact on their ecosystems'. Removing them, according to E.O. Wilson, 'results in a relatively significant shift in the composition of the [ecological] community'.

The finding that biocontrol success is typically determined by the effects of one to two species (Myers 1985; Denoth et al. 2002) is essentially indicating that there are keystone species in biological control. This broader definition of 'keystone' could be applied in a more specific way to biological control of exotic, invasive plants. *Aphthona* spp. Chevrolat, 1837. have exhibited an apparent keystone effect on *E. esula/virgata*, the underlying basis of which I have determined to be the insects providing avenues of ingress by soil borne plant pathogens (Caesar 2005), and thus soilborne root-infecting pathogens are themselves keystone species. Additionally, *U. scutellatus* is, to the knowledge of the present author, the first known application of nearest neighbor estimates of density to assess the potential impact of a candidate biological control agent.

Nearest neighbor-based spatial analysis has been used previously in plant disease ecology including studies of the spatial relationships among tree species caused by allelopathy (Nakamura et al. 1997). Other spatial analysis methods been used to

describe the effects on spatial patterns of plants infected with a smut fungus (Real & McElhany 1996). While the findings we present were based on few sites, it is important to indicate that sites that met the criteria to allow this study were rare. It was important for the purposes of the present study that there were sufficiently contiguous stands of *E. esula/virgata* with and without the occurrence of symptoms of the systemic autoecious rust *U. scutellatus* that were at the same time in close proximity. The work is based on extensive exploration in Hungary and Austria over several years (1991, 1995, 1998, 2001–2005) and at various portions of the respective growing seasons. It is also important to note that in following the impact of systemic rust diseases of *E. esula/virgata* beginning in 1992, there were a number of sites where rust symptoms were observed initially on dense stands of this species but in visits 1–4 years subsequent to the initial visit, dramatic stand reductions were seen. During the course of the present study this phenomenon occurred at a site near Gyongyos, Hungary. Thus, it is possible to conclude that *U. scutellatus* has high potential for biocontrol of *E. esula/virgata* in North America if it is shown to have a narrow host range and efficient means are identified to establish this rust in the field. It might be assumed that the complexity of the plant/herbivore/pathogen system ecological background of *E. esula/virgata* would hinder this study. However, that there are keystone species i.e., those which disproportionately affect the ecology of a species, would mitigate such a concern.

It has been presumed that the cumulative load of enemies (herbivores and plant pathogens) would be inversely proportionally or negatively determinative of invasiveness of a given exotic species (DeLoach 1995). This view that the cumulative enemy load (or lack thereof) would necessarily be determinative of invasion success is an apparent restating of the theory that pyramiding or stacking the natural enemy load against a target weed will increase success (Harris 1981). The findings in the present study, observations by the author in the field between 1992 and 2005 of lasting reductions of rust epidemic occurrence and observations by others of rapid stand density reduction following infection by *U. scutellatus* of the related euphorb *E. cyparissias* L. (Defago et al. 1985) provide broad indication this rust fungus is a keystone species affecting the density of *E. esula/virgata*. Paired rusted and rust free stands were used in the study to increase the likelihood that the background of enemy insects, diseases and deleterious rhizobacteria would be similar and thus be less likely to confound the possibility of attributing the differences in stand density to the occurrence or absence of rust disease. These various findings and field observations indicate the potential of *Uromyces scutellatus* to be an effective keystone biocontrol agent alongside the previously documented keystone pairing of soilborne fungi in combination with *Aphthona* spp.

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